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Prospects for Development of a Rotavirus Vaccine Against Rotavirus Diarrhea in Infants and Young Children

Albert Z. Kapikian, Jorge Flores, Yasutaka Hoshino, Karen Midthun, Mario Gorziglia, Kim Y. Green, Robert M. Chanock, Louis Potash, Stephen D. Sears, Mary Lou Clements, Neal A. Halsey, Robert E. Black, and Irene Perez-Schael

From the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; Flow Laboratories, McLean, Virginia; Johns Hopkins University, Baltimore, Maryland; and the Institute of Biomedicine, Central University of Venezuela, Caracas, Venezuela

Major advances have been made in elucidating the etiologic agents of severe infantile diarrhea, and it is clear that rotaviruses are the single most important etiologic agents. Progress in the development of rotavirus vaccine candidates has also moved swiftly with the "Jennerian" approach, in which a related live, attenuated rotavirus strain from a non-human host is used as the immunizing antigen. If this strategy is not effective against all rotavirus serotypes, reassortant rotaviruses hold great promise for the development of a multivalent vaccine. Field trials with the "Jennerian" approach vaccines are under way, and phase 1 trials with the reassortants have been initiated.

My assignment was to discuss several areas of rotavirus research, emphasizing (*1*) the global mortality and morbidity associated with rotavirus disease; (*2*) the availability of candidate experimental rotavirus vaccines; (*3*) a description of clinical trials with rotavirus vaccines; and (*4*) the prospects for the availability of a safe, inexpensive, and effective rotavirus vaccine in the not-too-distant future for routine use in the Expanded Programme on Immunization of the World Health Organization (WHO).

Morbidity and Mortality Associated with Diarrheal Disease

Diarrheal diseases are an important cause of morbidity in infants and young children in developed countries and a major cause of both morbidity and mortality in this same age group in developing countries [1, 2]. The scope of the problem in the United States was highlighted in the Cleveland Family Study, in which infectious gastroenteritis (considered nonbacterial) was the second most common disease, accounting for 16% of some 25,000 illnesses over a period of ~10 years (1948–1957) [3].

The toll from diarrheal disease in developing countries is staggering. An analysis of vital statistics submitted to WHO indicates that diarrheal diseases are responsible for a large proportion of the deaths in developing countries, accounting for as many as 15%–34% of all deaths in certain countries in a single year (table 1) [4]. Walsh and Warren estimated that in Asia, Africa, and Latin America 3–5 billion cases of diarrhea and 5–10 million deaths associated with diarrhea occur each year, ranking diarrheas first among infectious diseases in the categories of frequency of disease and mortality [5]. In addition, Snyder and Merson estimated from selected studies that in developing areas of Africa, Latin America, and Asia (excluding China), 744 million to 1 billion episodes of diarrhea and 4.6 million deaths due to diarrhea occur annually in children younger than five years of age [6]. This conservative estimate of mortality due to diarrheal disease is difficult to translate into comprehensible terms; however, when this estimate is broken down into smaller units, this annual estimate translates into ~12,600 deaths per day, or 525 deaths per hour.

The Role of Rotavirus in Diarrheal Disease

The search for etiologic agents of diarrhea has been especially compelling because of the immense toll it takes in the young in the developing countries of the world. Major advances have been made during the past 15 years in elucidating the etiologic agents of diarrhea. Before the early 1970s not a single viral agent was implicated as an important cause of infantile diarrhea [7]. Even viruses that grew efficiently

Please address requests for reprints to Dr. Albert Z. Kapikian, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892.

Table 1. Mortality from diarrheal diseases, 1968–1970.

Country, year (latest available data)	Diarrheal deaths		
	Rates per 100,000	No. of deaths	Percentage of all causes of death
Egypt, 1969	468.7	152,344	34.0
Guatemala, 1969	416.6	20,892	24.5
El Salvador, 1970	205.2	7,252	20.6
Mexico, 1970	150.6	72,652	15.0
Nicaragua, 1969	146.0	2,795	17.5
Honduras, 1970	140.9	3,640	17.9
Colombia, 1968	92.1	19,446	11.5
Mauritius, 1970	86.6	703	11.1
Ecuador, 1970	75.5	4,600	7.6
Costa Rica, 1970	69.1	1,221	10.6
Dominican Republic, 1970	61.2	2,489	10.0
Peru, 1969	60.5	7,962	8.0
Paraguay, 1970	56.5	1,347	11.2
Venezuela, 1970	56.5	5,536	8.1
Panama, 1970	47.3	679	6.6
Sri Lanka, 1968	46.2	5,530	5.8
Chile, 1969	43.2	4,134	4.8
Philippines, 1969	42.9	15,917	6.2
Portugal, 1970	29.3	2,827	3.1
Cuba, 1968	18.1	1,495	2.8
Thailand, 1970	18.0	6,105	2.7
Uruguay, 1970	11.3	326	1.2
Yugoslavia, 1970	9.9	2,016	1.1
Singapore, 1970	7.3	152	1.4
Israel, 1970	6.4	187	0.9
Italy, 1970	5.4	2,885	0.6
Greece, 1970	4.9	438	0.6
Japan, 1970	4.5	4,742	0.7
Spain, 1969	4.0	1,345	0.5
Hungary, 1970	3.3	346	0.3

NOTE. Table is reprinted with permission from the WHO *Weekly Epidemiological Record* [4].

in the enteric tract, such as the echoviruses—which were discovered as a direct result of the tissue culture era and which held promise as important causative agents of gastroenteritis—could not be associated etiologically. Bacterial and parasitic agents known at that time also failed to fill this etiologic vacuum, since they were associated with only a small percentage of the hospitalizations for diarrhea among infants and young children [8].

The discovery in 1972 of the 27-nm Norwalk virus and of its association with epidemic viral gastroenteritis in older children and adults followed by the discovery in 1973 of the 70-nm human rotavirus and its association with acute gastroenteritis of infants and young children represent major recent advances in the long and elusive search for etiolgic agents of acute infectious nonbacterial gastroenteritis [9, 10] (figure 1). Both viruses were discovered with the use of electron microscopy: Norwalk virus was visualized by immune electron microscopy, in which convalescent-phase sera from individuals with diarrheal illness were reacted with an infectious stool inoculum; rotavirus was visualized by examination of thin sections of duodenal tissue from infants with diarrheal illness. Rotaviruses were soon detected by electron microscopic examination of feces and later by various immunoassays [2, 12].

Rotaviruses are the single most important etiologic agents of severe diarrhea of infants and young children in developed and developing countries [2]. They are associated with 35%–50% of severe diarrheal disease in children younger than two years of age. Two long-term cross-sectional studies of hospitalizations due to diarrheal illness—one in Washington, D.C., from 1974 to 1982 and the other in Yamagata City, Japan, from 1974 to 1981—clearly demonstrate the important role of these agents [13, 14]. In the studies from the United States and Japan, 34.5% of 1,537 children and 45% of 1,910 children, respectively, shed rotavirus in feces. The role of other agents was negligible.

It is also clear from a number of studies that rotaviruses are responsible for a significant proportion of the serious diarrheal illnesses in countries in which mortality due to diarrhea is high [1]. For example, in Bangladesh in a one-year study of patients coming to a treatment center with diarrheal illnesses of varying severity, rotaviruses were the most frequently identified pathogens in children younger than two years of age; 46% were rotavirus-positive, whereas 28% shed enterotoxigenic *Escherichia coli* [15]. In patients older than two years of age, bacterial pathogens were identified more frequently than rotaviruses.

The capacity of rotaviruses to induce severe dehydrating illness with greater frequency than that induced by other agents has been demonstrated in several reports. As shown in table 2, summary projections from various studies in infants and young children indicate that although rotaviruses were associated with only 6% of all diarrheal episodes, they accounted for a disproportionately large number of life-threatening dehydrating diarrheal illnesses [16].

Because of the major impact of diarrheal illnesses

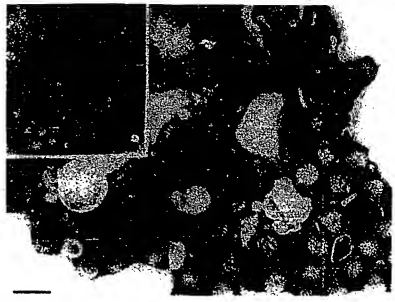

Figure 1. *A*: A group of Norwalk virus particles observed after incubation of 0.8 mL of stool filtrate (prepared from the stool of a volunteer administered the Norwalk agent) with 0.2 mL of a 1:5 dilution of the volunteer's prechallenge serum and further preparation for electron microscopy. The quantity of antibody on these particles was rated as 1+ (bar = 100 nm). Reprinted with permission from the *Journal of Virology* [9]. *B*: Human rotavirus particles observed in a stool filtrate (prepared from a stool of an infant with gastroenteritis) after incubation with PBS and further preparation for electron microscopy. The particles appear to have a double-shelled capsid. Occasional "empty" particles are seen (bar = 100 nm). Reprinted with permission from *Science* [11].

caused by rotavirus in infants and young children, intensive efforts are under way to develop a rotavirus vaccine [1]. The aim is to prevent severe rotavirus diarrhea during the first two years of life. Evidence from animal studies indicates that local intestinal immunity plays the major role in resistance to rotavirus disease [17]. Therefore, it appears that an orally administered attenuated live virus vaccine would be more effective than a parenteral vaccine.

I would like to briefly highlight some characteristics of the virus that are important to an understanding of vaccine strategies. Rotaviruses have a distinctive morphologic appearance by negative-stain electron microscopy (figure 1, B). Complete particles have a double-layered capsid and measure 70 nm in diameter. The name *rotavirus* is derived from the Latin word *rota* (wheel) and was suggested because the sharply defined circular outline of the outer capsid gives the appearance of the rim of a wheel placed on short spokes radiating from a wide hub [18]. The rotavirus genome contains 11 segments of double-stranded RNA [2]. Segments 1, 2, and 6 code for inner-capsid polypeptides VP1, VP2, and VP6, respectively. VP6 contains a domain for the common group antigen, which is shared by most human and animal rotaviruses, and a separate domain for the subgroup antigen, which further distinguishes strains. RNA segments 4 and 8 or 9 encode the major outer-capsid polypeptides VP3 and VP7, respectively. The latter is the major neutralization antigen, whereas the former—which is also associated with neutralization—is responsible for protease-enhanced infectivity and plaque formation in tissue culture as well as for hemagglutination of certain strains of rotavirus [2].

The development of techniques for propagation of human rotaviruses in cell culture has made it possible to identify rotavirus serotypes by conventional assays [19, 20]. The criterion for establishing a distinct serotype is a >20-fold difference in the reciprocal serum antibody titer between a candidate strain

Table 2. Maximum impact of rotavirus immunization on diarrhea morbidity and mortality rates among children under five years of age in developing countries, assuming 100% vaccine efficacy, 100% program coverage, and an average age at full immunization of six months.

Age (mo)	Proportion of diarrhea episodes		Proportion of diarrhea deaths	
	Caused by rotavirus (%)	Averted by rotavirus immunization (%)	Caused by rotavirus (%)	Averted by rotavirus immunization (%)
0–5	8	0	12	0
6–23	10	10	30	30
24–59	1	1	5	5
0–59	6	5	20	16

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and each of the established serotypes. At present, four epidemiologically important human rotavirus serotypes have been identified [21, 22].

Candidate Rotavirus Vaccines

Edward Jenner's concept of vaccinating humans against smallpox with attenuated infectious material from a non-human host has been adapted for the development of a human rotavirus vaccine [1]. The "Jennerian" approach to rotavirus vaccination was suggested by the finding that human and animal rotaviruses share a common group antigen that makes them indistinguishable in various serologic assays (e.g., complement fixation, immunofluorescence, and ELISA) with hyperimmune animal and infection-derived animal or human sera [2, 18, 23]. This property is mediated by the major inner-capsid protein (VP6) encoded by gene segment 6 [2]. The potential effectiveness of the Jennerian approach to rotavirus vaccination was demonstrated in gnotobiotic, colostrum-deprived calves: after inoculation in utero with bovine rotavirus (NCDV) (a serotype 6 rotavirus [22]), calves were protected against disease following challenge at birth with human rotavirus type 1 [24]. In addition, following the in utero inoculation, most calves developed serum-neutralizing antibodies to heterotypic rotavirus serotypes 1, 2, and 3 [25]. The feasibility of this approach was later also confirmed in studies in piglets [26].

Bovine Rotavirus NCDV (Lincoln [RIT 4237]) Strain

The most extensively tested human rotavirus vaccine (RIT 4237) is derived from the cold-adapted bovine rotavirus NCDV (Lincoln) strain [27, 28]. This attenuated vaccine was passaged 147 times in bovine embryonic kidney cells and seven times in primary African green monkey kidney cells. A protection rate of 88% against clinically significant rotavirus diarrhea was observed in 178 Finnish infants 8–11 months of age following a single oral dose of the RIT 4237 vaccine [27]. In a later study, a protective efficacy of 82% was observed in 331 Finnish infants 6–12 months of age following two oral doses [28]. In this study, the vaccine appeared to protect against serotype 1–induced diarrhea. The observation that the vaccine was not protective against mild rotavirus illness was not unexpected, since reinfections occur frequently under natural conditions in both children and adults [29, 30]. However, primary infection does appear to decrease the severity of illness during reinfection. Thus, the realistic goal of a vaccine is to prevent serious rotavirus disease rather than rotavirus infection or mild rotavirus illness. Although the RIT 4237 vaccine appears quite promising, it needs to be further evaluated with regard to (*1*) its antigenicity under normal field conditions (e.g., in neonates with high levels of maternal antibodies; in combination with oral poliovirus vaccine; or after its administration without an agent to buffer stomach acid, since it is acid-labile [31]); (*2*) its efficacy in developing countries; and (*3*) its cost-effectiveness, since it is administered undiluted for maximum antigenicity.

Rhesus Rotavirus Strain MMU 18006

In several collaborative studies, we are evaluating another animal rotavirus—the rhesus rotavirus strain MMU 18006—as a candidate rotavirus vaccine [32–40]. This strain was isolated from the stool of a 3½-month-old rhesus monkey with diarrhea [41]. The vaccine strain has been passaged nine times in primary or secondary monkey kidney cell cultures and seven times in DBS-FRhL2 cells (a semicontinuous diploid strain of fetal rhesus monkey lung cells developed as a possible cell substrate for vaccine production by the Office of Biologics, Food and Drug Administration [42]). The rhesus rotavirus strain has several attributes that make it attractive for clinical evaluation: e.g., its apparent restriction in humans (since it has not been recovered in humans under natural conditions); its shared neutralization specificity with human rotavirus type 3; and its growth to high titer in DBS-FRhL2 cells, an important consideration since adventitious agents are frequently found in primary monkey kidney cell culture [33].

Studies of the reactogenicity and antigenicity of this vaccine candidate have been described in detail elsewhere [1, 32–40] and will not be recapitulated extensively here. In summary: (*1*) the vaccine was shown to be safe and antigenic after oral administration of 10^4 pfu (undiluted) or 10^3 pfu (1:10 dilution) of a buffered dose in early studies that began in adult volunteers and proceeded in stepwise fashion to children and infants [1, 32–40]; (*2*) in a direct comparison with the RIT 4237 vaccine in six-to-eight-month-old Finnish infants, the rhesus rotavirus vaccine (10^5 pfu) induced significant febrile reactions in 64% and watery stools in 20% of vaccinees [36]. Similar reac-

tions were observed in other locations in the United States and Sweden [34, 35, 37, 40]. The rhesus rotavirus vaccine was more antigenic than the RIT 4237 vaccine [1, 36]; (*3*) lowered doses of rhesus rotavirus (10^4 or 10^3 pfu) were nonreactogenic in four- to 10-month-old infants in Venezuela, and the 10^4 pfu dose was quite antigenic [39]; (*4*) the difference in reactogenicity may have been due to the significantly higher prevaccination titers of serum antibody to rhesus rotavirus in the United States and Venezuelan children in comparison to titers in the Finnish study group, a finding suggesting that such antibody attenuated the clinical response (but did not interfere with the antigenicity of the vaccine) [1, 34].

Because of these observations, and of the knowledge that in developing countries rotavirus diarrhea can occur in children younger than six months of age and that exposure to health care providers is most frequent during the first few months of life (which would thus enhance the likelihood of programmed vaccination), we evaluated the vaccine in one- to four-month-old infants, anticipating that the presence of maternal antibody might attenuate the reactogenicity of the vaccine [39, 43]. Our goal was to identify a dose that could stimulate a silent immunizing infection under the cover of passively acquired circulating (via the placenta) or local (via the breast milk) rotavirus antibodies. A dose of vaccine of 10^4 pfu was shown to be nonreactogenic and antigenic in one- to four-month-old children in Venezuela [39]. We therefore initiated several field trials employing a 10^4 pfu dose of rhesus rotavirus vaccine. As shown in table 3, >600 infants younger than five months of age are enrolled in double-blind field trials at various locations in the United States and elsewhere. The vaccine is not currently being administered to children after the age of five months since most passively acquired serum antibody is lost by this age and the reactogenicity of the vaccine would thus be increased. The results of these field trials are awaited with great interest.

Table 3. Clinical trials of rhesus rotavirus vaccine candidate.

Institution (investigators)	No. of infants enrolled	Age of enrollees (mo)
University of Maryland (Rennels, Losonsky, Levine)	30	2–11
Institute of Biomedicine, Caracas, Venezuela (Perez-Schael, Flores)	240	1–10
Vanderbilt University, Tennessee (Wright)	30	4–12
Marshall University, West Virginia (Anderson, Belshe)	30	4–12
University of Umeå, Sweden (Gothefors, Wadell)	106*	4–12
University of Tampere, Finland (Vesikari)	200	2–5
Johns Hopkins University, Maryland/ White River and Tuba City, Arizona (Santosham, Sack)	210	2–5
University of Rochester, New York (Christy, Dolin)	176	2–4
King Edward Medical College, Lahore, Pakistan† (Jalil)	74	1.5
University of Umèa, Sweden (Gothefors, Wadell)	40	1–4

* Dose of 10^5 pfu; all others were 10^4 pfu.
† Phase 1 study.

Reassortant Rotaviruses

If the Jennerian concept fails, and these vaccines from non-human hosts do not provide protection against each of the four epidemiologically important serotypes, another approach to the development of an attenuated rotavirus vaccine that takes advantage of the propensity of rotaviruses to undergo gene reassortment with high efficiency during coinfection can be implemented. Thus, rhesus rotavirus can be used as a donor of attenuating genes that can be transferred during coinfection with a specific serotype of human rotavirus (under selective pressure of antibodies to the animal rotavirus) to form a hybrid or reassortant rotavirus that possesses only the major neutralization protein (VP7) of a human rotavirus belonging to serotype 1, 2, or 4 and the remaining 10 genes from the rhesus rotavirus (figure 2) [44, 45]. Such single-gene substitution reassortants are available for serotypes 1, 2, and 4 human rotaviruses. In a similar way, single-gene substitution reassortants have been prepared for each of the four human rotavirus serotypes with the remaining 10 genes from bovine rotavirus UK [44, 45]. In addition, since VP3 (another outer-capsid protein) also induces the production of neutralizing antibodies that can protect against rotavirus disease in animal models, it might be feasible to form a reassortant vaccine that possesses a broadly reactive VP3 and the VP7 of a human rotavirus, if such a cross-reactive VP3 can be found in an attenuated strain [46].

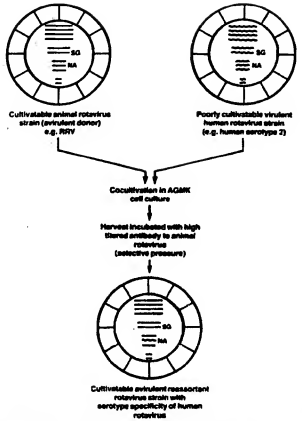

Figure 2. Production of a reassortant rotavirus vaccine. RRV = rhesus rotavirus; AGMK = African green monkey kidney. Genes and coding subgroup (SG) antigen and major neutralization antigen (NA) are indicated. Adapted from [34].

We have recently initiated phase 1 trials with two reassortant rotaviruses in adult volunteers at the Francis Scott Key Hospital in Baltimore. The two rotaviruses are the D (human rotavirus serotype 1) × RRV (rhesus rotavirus) reassortant with the neutralization specificity (VP7) of human rotavirus serotype 1 and the DS-1 (human rotavirus serotype 2) × RRV reassortant with the neutralization specificity (VP7) of human rotavirus serotype 2. Initially, two volunteers with high levels of plaque-reduction neutralization (PRN) serum antibody (1:640) to the D × RRV reassortant were administered 10^4 pfu of this reassortant orally after administration of $NaHCO_3$, as a buffer. Neither volunteer became ill. Subsequently, eight additional volunteers with the lowest available PRN serum antibody (1:160) to the reassortant were administered the same inoculum. Again, none developed illness.

Similar studies were carried out with a DS-1 × RRV reassortant in two volunteers with high levels of prechallenge PRN antibody (1:160 or 1:640) to this reassortant. Neither volunteer became ill. Subsequently, 14 volunteers with little, if any, prechallenge PRN serum antibody (<1:80) to this reassortant were given the reassortant; none developed diarrheal illness. We are planning to carry out studies stepwise with these reassortant viruses in children three to 12 years of age. If significant reactions are not observed and if the vaccine proves to be antigenic, we plan to vaccinate progressively younger seropositive infants in a stepwise fashion until we are vaccinating newborn infants. If the vaccine is safe and antigenic, our goal is to routinely vaccinate the newborn to five-month-old age group, with the ideal regimen being a single dose of vaccine at birth or at 6 weeks of age. We are also considering a four-cell field trial in infants younger than five months of age: one group would receive the D × RRV reassortant (serotype 1); another, the DS-1 × RRV reassortant (serotype 2); a third, these two reassortants plus RRV (serotype 3) in a multivalent vaccine; and a fourth, a placebo. This study should help elucidate the role of homotypic and heterotypic immunity.

Other Cell Culture Approaches

Rotavirus strains obtained from neonates undergoing asymptomatic infections may also hold promise as vaccine candidates since they may represent naturally occurring attenuated strains [47]. Each of the four rotavirus serotypes has been isolated from newborns with asymptomatic infections [47]. One strain recovered from asymptomatic neonates within the first 14 days of life induced significant protection against serious rotavirus disease for up three years [48]. However, infection with this naturally occurring strain failed to protect against rotavirus infection and mild or moderate diarrheal illness; nine of 24 neonatally infected infants and nine of 20 nonneonatally infected children developed mild or moderate illnesses associated with rotavirus infections during the postneonatal period. However, all eight severe diarrheal episodes, including three that resulted in hospitalization, occurred in the nonneonatally infected group. Thus, prior infection with a naturally occurring wild-type rotavirus during the

neonatal period induced protection against severe rotavirus illness but not against reinfection or mild illness.

A neonatal strain could also be used to construct a reassortant strain comprising 10 genes from a neonatal strain and a single gene (VP7) from different virulent rotavirus serotypes. This approach could yield attenuated strains against specific serotypes, since the nursery strains, which appear to be naturally attenuated, might be donors of the attenuating gene(s), whereas the outer-capsid serotype-specific protein VP7 would be contributed by the virulent strain [47].

Attenuation of virulent human rotavirus strains might also be achieved by cold adaptation [49].

Thus, important advances are being made in the development of a rotavirus vaccine aimed at preventing a major cause of illness in infants and young children in developed countries and of illness and death in developing countries. Perhaps the concept developed by Edward Jenner for prevention of smallpox can be applied successfully some 200 years later for prevention of another important disease—rotaviral diarrhea of infants and young children.

References

1. Kapikian AZ, Flores J, Hoshino Y, Glass RI, Midthun K, Gorziglia M, Chanock RM. Rotavirus: the major etiologic agent of severe infantile diarrhea may be controllable by a "Jennerian" approach to vaccination. J Infect Dis 1986;153:815–22
2. Kapikian AZ, Chanock RM. Rotaviruses. In: Fields BN, ed. Virology. New York: Raven Press, 1985:863–906
3. Dingle JH, Badger GF, Jordan WS. Illness in the home: a study of 25,000 illnesses in a group of Cleveland families. Cleveland: Western Reserve University Press, 1964:19–32
4. World Health Organization. Mortality due to diarrheal diseases in the world. Weekly Epidemiological Record 1973; 43:409–16
5. Walsh JA, Warren KS. Selective primary health care: an interim strategy for disease control in developing countries. N Engl J Med 1979;301:967–74
6. Snyder JD, Merson MH. The magnitude of the global problem of acute diarrheal disease: a review of active surveillance data. Bull WHO 1982;60:605–13
7. Sidwell RW. Overview of viral agents in pediatric enteric infections. Pediatr Infect Dis 1986;5:S44–5
8. Yow MD, Melnick JL, Blattner RJ, Stephenson WB, Robinson NM, Burkhardt MA. The association of viruses and bacteria with infantile diarrhea. Am J Epidemiol 1970; 92:33–9
9. Kapikian AZ, Wyatt RG, Dolin R, Thornhill TS, Kalica AR, Chanock RM. Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. J Virol 1972;10:1075–81
10. Bishop RF, Davidson GP, Holmes IH, Ruck BJ. Virus particles in epithelial cells of duodenal mucosa from children with viral gastroenteritis. Lancet 1973;2:1281–3
11. Kapikian AZ, Kim HW, Wyatt RG, Rodriguez WJ, Ross S, Cline WL, Parrott RH, Chanock RM. Reovirus-like agent in stools: association with infantile diarrhea and development of serologic tests. Science 1974;185:1049–53
12. Yolken RH. Enzyme immunoassays for detecting human rotavirus. In: Tyrrell DAJ, Kapikian AZ, eds. Virus infections of the gastrointestinal tract. New York: Marcel Dekker, 1982:51–74
13. Brandt CD, Kim HW, Rodriguez WJ, Arrobio JO, Jeffries BC, Stallings EP, Lewis C, Miles AJ, Chanock RM, Kapikian AZ, Parrott RH. Pediatric viral gastroenteritis during eight years of study. J Clin Microbiol 1983;18:71–8
14. Konno T, Suzuki H, Katsushima N, Imai A, Tazawa F, Kutsuzawa T, Kitaoka S, Sakamoto M, Yazaki N, Ishida N. Influence of temperature and relative humidity on human rotavirus infection in Japan. J Infect Dis 1983;147:125–8
15. Black RE, Merson MH, Mizamur Rahman ASM, Yunus M, Alim ARMA, Huq I, Yolken RH, Curlin GT. A two-year study of bacterial, viral and parasitic agents associated with diarrhea in rural Bangladesh. J Infect Dis 1980;142:660–4
16. De Zoysa I, Feachem RG. Interventions for the control of diarrheal diseases among young children: rotavirus and cholera immunization. Bull WHO 1985;63:569–83
17. Snodgrass DR, Wells PW. Passive immunity in rotaviral infections. J Am Vet Med Assoc 1978;173:565–8
18. Flewett TH, Bryden AS, Davies H, Woode GN, Bridger JC, Derrick JM. Relationship between virus from acute gastroenteritis of children and newborn calves. Lancet 1974;2:61–3
19. Sato K, Inaba Y, Shinozaki T, Fujii R, Matsumoto M. Isolation of human rotavirus in cell cultures. Arch Virol 1981;69:155–60
20. Urasawa T, Urasawa S, Taniguchi K. Sequential passages of human rotavirus in MA-104 cells. Microbiol Immunol 1981;25:1025–35
21. Wyatt RG, Greenberg HB, James WD, Pittman AL, Kalica AR, Flores J, Chanock RM, Kapikian AZ. Definition of human rotavirus serotypes by plaque reduction assay. Infect Immun 1984;37:110–15
22. Hoshino Y, Wyatt RG, Greenberg HB, Flores J, Kapikian AZ. Serotypic similarity and diversity of rotaviruses of mammalian and avian origin as studied by plaque-reduction neutralization. J Infect Dis 1984;149:694–702
23. Kapikian AZ, Cline WL, Kim HW, Kalica AR, Wyatt RG, Van Kirk DH, Chanock RM, James HD Jr, Vaughn AL. Antigenic relationships among five reovirus-like (RVL) agents by complement fixation (CF) and development of a new substitute CF antigen for the human RVL agent of infantile gastroenteritis. Proc Soc Exp Biol Med 1976; 152:535–9
24. Wyatt RG, Mebus CA, Yolken RH, Kalica AR, James HD Jr, Kapikian AZ, Chanock RM. Rotaviral immunity in gnotobiotic calves: heterologous resistance to human virus induced by bovine virus. Science 1979;203:548–50
25. Wyatt RG, Kapikian AZ, Mebus CA. Induction of cross-reactive serum neutralizing antibody to human rotavirus

in calves after in utero administration of bovine rotavirus. J Clin Microbiol 1983;18:505–8
26. Zissis G, Lambert JP, Marbehant P, Marissens D, Lobmann M, Charlier P, Delem A, Zygraich N. Protection studies in colostrum-deprived piglets of a bovine rotavirus vaccine candidate using human rotavirus strains for challenge. J Infect Dis 1983;148:1061–8
27. Vesikari T, Isolauri E, D'Hondt E, Delem A, Andre FE, Zissis G. Protection of infants against rotavirus diarrhea by RIT 4237 attenuated bovine rotavirus strain vaccine. Lancet 1984;1:977–81
28. Vesikari T, Isolauri E, Delem A, D'Hondt E, Andre F, Beards GM, Flewett TH. Clinical efficacy of the RIT 4237 live attenuated bovine rotavirus vaccine in infants vaccinated before a rotavirus epidemic. J Pediatr 1985;107:189–94
29. Gurwith M, Wenman W, Hinde D, Feltham S, Greenberg H. A prospective study of rotavirus infection in infants and young children. J Infect Dis 1981;144:218–24
30. Kim HW, Brandt CD, Kapikian AZ, Wyatt RG, Arrobio JO, Rodriguez WJ, Chanock RM, Parrott RH. Human reovirus-like agent (HRVLA) infection: occurrence in adult contacts of pediatric patients with gastroenteritis. JAMA 1977;238:404–7
31. Vesikari T, Isolauri E, D'Hondt E, Delem A. Increased "take" rate of oral rotavirus vaccine in infants after milk feeding. Lancet 1984;2:700
32. Wyatt RG, Kapikian AZ, Hoshino Y, Flores J, Midthun K, Greenberg HB, Glass RI, Askaa J, Levine MM, Black RE, Clements ML, Potash L, London WT. Development of rotavirus vaccines. In: Control and eradication of infectious diseases. An international symposium. Washington, DC: Pan American Health Organization, 1985:17–28
33. Kapikian AZ, Midthun K, Hoshino Y, Flores J, Wyatt RG, Glass RI, Askaa J, Nakagomi O, Nakagomi T, Chanock RM, Levine MM, Clements ML, Dolin R, Wright PF, Belshe RB, Anderson EL, Potash L. Rhesus rotavirus: a candidate vaccine for prevention of human rotavirus disease. In: Lerner RA, Chanock RM, Brown F, eds. Vaccines 85. Molecular and chemical basis of resistance to parasitic, bacterial, and virus diseases. New York: Cold Spring Harbor Laboratory, 1985:357–67
34. Kapikian AZ, Hoshino Y, Flores J, Midthun K, Glass RI, Nakagomi O, Nakagomi T, Chanock RM, Potash L, Levine MM, Dolin R, Wright PF, Belshe RE, Anderson EL, Vesikari T, Gothefors L, Wadell G, Perez-Schael I. Alternative approaches to the development of a rotavirus vaccine. In: Holmgren J, Lindberg A, Mollby R, eds. Development of vaccines and drugs against diarrhea. 11th Nobel Conference, Stockholm 1985. Lund, Sweden: Studentlitteratur, 1986:152–214
35. Anderson EL, Belshe RB, Bartram J, Crookshanks-Newman F, Chanock RM, Kapikian AZ. Evaluation of rhesus rotavirus vaccine (MMU 18006) in infants and young children. J Infect Dis 1986;153:823–31
36. Vesikari T, Kapikian AZ, Delem A, Zissis G. A comparative trial of rhesus monkey (RRV-I) and bovine (RIT 4237) oral rotavirus vaccines in young children. J Infect Dis 1986; 153:832–9
37. Losonsky GA, Rennels MB, Kapikian AZ, Midthun K, Ferra PJ, Fortier DN, Hoffman KM, Baig A, Levine MM. Safety, infectivity, transmissibility, and immunogenicity of rhesus rotavirus vaccine (MMU 18006) in infants. Pediatr Infect Dis 1986;5:25–9
38. Christy C, Madore HP, Treanor JJ, Pray K, Kapikian AZ, Chanock RM, Dolin R. Safety and immunogenicity of live attenuated rhesus monkey rotavirus vaccine. J Infect Dis 1986;154:1045–7
39. Perez-Schael I, Gonzalez M, Daoud N, Perez M, Soto G, Garcia D, Kapikian AZ, Flores J. Reactogenicity and antigenicity of the rhesus rotavirus vaccine in Venezuelan children. J Infect Dis 1987;155:334–7
40. Wright PF, Tajima T, Thompson J, Kokubun K, Kapikian A, Karzon DT. Candidate rotavirus vaccine (rhesus rotavirus strain) in children: an evaluation. Pediatrics 1987; 80:473–80
41. Stuker G, Oshiro L, Schmidt NL. Antigenic comparisons of two new rotaviruses from rhesus monkeys. J Clin Microbiol 1980;11:202–3
42. Wallace RE, Vasington PJ, Petricciani JC, Hopps HE, Lorenz DE, Kadanka Z. Development of a diploid cell line from fetal rhesus monkey lung for virus vaccine production. In Vitro 1973;8:323–32
43. Halsey N, Galazka A. The effectiveness of DPT and oral poliomyelitis immunization initiated from birth to 12 weeks of age. Bull WHO 1985;63:1151–69
44. Midthun K, Greenberg HB, Hoshino Y, Kapikian AZ, Wyatt RG, Chanock RM. Reassortant rotaviruses as potential live rotavirus vaccine candidates. J Virol 1985;53:949–54
45. Midthun K, Hoshino Y, Kapikian AZ, Chanock RM. Single gene substitution rotavirus reassortants containing the major neutralization protein (VP7) of human rotavirus serotype 4. J Clin Microbiol 1986;24:822–6
46. Hoshino Y, Sereno MM, Midthun K, Flores J, Kapikian AZ, Chanock RM. Independent segregation of two antigenic specificities (VP3 and VP7) involved in neutralization of rotavirus infectivity. Proc Natl Acad Sci USA 1985;82: 8701–4
47. Hoshino Y, Wyatt RG, Flores J, Midthun K, Kapikian AZ. Serotypic characterization of rotaviruses derived from asymptomatic human neonatal infections. J Clin Microbiol 1985;21:425–30
48. Bishop RF, Barnes GL, Cipriani E, Lund JS. Clinical immunity after neonatal rotavirus infection. A prospective longitudinal study in young children. N Engl J Med 1983; 309:72–6
49. Matsuno S, Murekami S, Takaji M, Hayashi M, Inouye S, Hasegawa A, Fukai K. Cold adaptation of human rotavirus. Virus Research 1987;7:273–80